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**MECHANISMS OF REDUCED
EFFECTS OF LOOP DIURETICS
IN HEALTHY VOLUNTEERS AND
IN PATIENTS WITH RENAL
DISEASE**

by

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ABSTRACT

MECHANISMS OF REDUCED EFFECTS OF LOOP DIURETICS IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RENAL DISEASE.

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Although most patients respond well to loop diuretics, poor response is sometimes a problem and some underlying mechanisms were addressed in this study.

The renal response to continuous infusion of furosemide was investigated in eight healthy volunteers during controlled isotonic dehydration and after full restoration of volume losses. A rapidly reversible acute tolerance developed in parallel with dehydration and activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS). Dehydration also reduced the renal clearance of furosemide substantially, but only decreased the urinary delivery rate of the drug (the principal determinant of the diuretic effect) to a minimal extent.

Delayed tolerance to an i.v. bolus dose of furosemide was found in 12 healthy volunteers after 1 week of oral furosemide treatment with and without angiotensin converting enzyme inhibition. No pharmacokinetic changes were seen. This type of tolerance was not related to dehydration or activation of RAAS. Thus, the induced decrease in renal sensitivity to furosemide was probably due to an intrarenal (structural?) adaptation.

The pharmacokinetics and pharmacodynamics of piretanide were studied in six healthy volunteers and 22 patients with chronic renal failure (glomerular filtration rate 1 - 28 ml/min). Poor response to the diuretic action of the drug was found in the patients. This was entirely due to a decrease in the fraction of piretanide excreted unchanged in the urine, and the renal sensitivity to the drug was normal. Multiple daily doses of piretanide of maximally 24 mg are recommended for optimal efficiency in renal failure.

Substantial changes in pharmacokinetics of furosemide were found after manipulation of plasma albumin in five patients with nephrosis, while the urinary delivery of the drug scarcely changed. Neither the induced alterations in proteinuria nor those in plasma volume influenced the renal sensitivity to furosemide significantly.

Some methodological observations proved to be of significance. Creatinine was found to be an unreliable marker of GFR because of its substantial tubular secretion and reabsorption, both of which were related to the degree of hydration. Likewise, lithium was considered an unreliable marker of proximal tubular reabsorption, since there were reasons to suspect furosemide-sensitive distal lithium reabsorption.

Key words: Loop diuretic, angiotensin converting enzyme inhibitor, furosemide, piretanide, lisinopril, pharmacokinetics, pharmacodynamics, renal function, healthy volunteers, chronic renal failure, nephrotic syndrome, sympathetic nervous system, renin-angiotensin-aldosterone system, plasma volume.

To Karin

LIST OF PUBLICATIONS

This thesis is based on studies reported in the publications listed below. They will be referred to in the following text by their Roman numerals.

- I:A. Sjöström P, Odling B, Beermann B, Hammarlund-Udenaes M: On the mechanism of acute tolerance to furosemide diuresis. Scand J Urol Nephrol. In press 1988; Vol. 22.
- I:B. Sjöström P, Odling B, Beermann B, Hammarlund-Udenaes M: Changes in furosemide pharmacokinetics induced by dehydration. In: Diuretics: Basic, Pharmacological, and Clinical Aspects. Ed.: Andreucci VE, Dal Canton A. Martinus Nijhoff, Boston, USA, 1987;107-109.
- II. Sjöström P, Beermann B, Odling B: Delayed tolerance to furosemide diuresis. Influence of angiotensin converting enzyme inhibition by lisinopril. Scand J Urol Nephrol. Accepted for publication.
- III. Sjöström P, Beermann B, Odling B: Pharmacokinetic-pharmacodynamic relationship of piretanide in healthy and uremic subjects. Determinants of the diuretic effect of a loop diuretic. Scand J Urol Nephrol 21:55-64, 1987.
- IV. Sjöström P, Odling B, Beermann B, Karlberg B: Pharmacokinetics and effects of furosemide in patients with nephrosis. Influence of plasma volume expansion with albumin and dextran. In manuscript.
- V. Sjöström P, Odling B, Beermann B: Is lithium an ideal marker of proximal tubular sodium reabsorption? In: Diuretics II: Chemistry, Pharmacology and Clinical Applications. Ed.: Puschett JB, Greenberg A. Elsevier Science Publishing Co., New York City, 1987;215-217.
- VI. Sjöström P, Odling B, Wolgast M. Extensive tubular secretion and reabsorption of creatinine in humans. Scand J Urol Nephrol. In press 1988; Vol. 22.

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INTRODUCTION

Loop diuretics are used in a variety of disorders involving fluid retention, e.g. chronic heart failure, cirrhosis of the liver, nephrotic syndrome, chronic renal disease and idiopathic oedema. Some of the patients respond poorly to these agents. This poor response has been called resistance, the cause of which may differ between the various disorders (19, 96).

The response to loop diuretics has also been found to vary in healthy volunteers (4, 16, 17), and a decrease in response or development of tolerance to the diuretic has been observed in various experimental settings (17, 43, 51, 58).

Some of the physiological and pharmacokinetic mechanisms underlying resistance to loop diuretics may be connected with those underlying tolerance, and these phenomena are therefore discussed together in this review.

Regulation of salt and water homeostasis

Sodium is the principal osmotic component of the extracellular fluid and the main determinant of the extracellular fluid volume (ECV). Many physiological systems are involved in the preservation of salt and ECV balance. They differ in efficacy, time of onset and duration and they are able to amplify and replace each other. It is reasonable to assume that these homeostatic mechanisms may interfere with the response to a loop diuretic.

Intrarenal mechanisms: The kidney is the main effector organ in the preservation of ECV. Taking part in this function are several intrarenal mechanisms for regulation of the glomerular filtration rate (GFR) and tubular reabsorption.

In the proximal tubule about 65 % of the filtered salt and water are reabsorbed isototically. In the loop of Henle an additional 25 % of the filtered sodium is reabsorbed - the main part in the thick ascending limb (TALH) - and in the distal tubule and collecting duct about 10 %, and ultimately regulates the water and electrolyte excretion (Table I, Fig. 1). The primary force for all active or secondary active sodium and chloride reabsorption is the sodium gradient generated by the Na^+/K^+ pump ($\text{Na}^+/\text{K}^+-\text{ATPase}$; 116) in

the basolateral membrane of the tubular cells (Fig. 2).

The tubular reabsorption varies within each segment and there is marked heterogeneity between and also within nephrons, an arrangement that is important in the regulation of salt and water balance (Jacobson HR and Kokko JP, p 531 in ref. 115).

At the same time, and also important to the balance, there is constancy in the fraction reabsorbed in a tubular segment, unless the tubular flow rate (49 75), the peritubular milieu (21, 112) or the influence of hormonal or neuronal factors is altered. This is best studied and verified in the proximal tubule and is referred to as the glomerulo-tubular balance (47, 119). Changes in ECV, especially volume expansion, when the plasma albumin concentration (oncotic pressure) decreases, disrupt the glomerulo-tubular balance, so that less salt and water is reabsorbed in the proximal tubules.

Another important factor in the steady state balance between glomerular filtration and tubular reabsorption is the tubulo-glomerular feedback mechanism (113, 125), which counteracts variations in the intratubular flow rate by changing GFR. The feedback loop starts in the macula densa, which senses the luminal salt concentration. This concentration is dependent on the tubular flow rate and reabsorption proximal to the macula densa. An increasing salt concentration triggers a vasomotor reaction in the afferent arteriole, in

Table I

Tubular sodium reabsorption, intraluminal concentrations and electrical potential. Varying values can be found (115).

	PCT	*	HL	*	DT & CD	*
	(Urine)					
% filtered Na ⁺ reabsorbed	65		25		9	
remaining		35		10		1
Segmental EF (%)	35		29		10	
Nephron EF (%)						1
Tubular fluid						
Na ⁺ conc. (mM)		140		50-130		5-135
Osmolality (mOsm)		300		1000 - 100		100-1000
Potential (mV)	-4	to +2	to 0	to +10	to -50	to 0

PCT = Proximal convoluted tubule, HL = Henle's loop, DT = Distal tubule, CD = Collecting duct, EF = excretion fraction, and
 * = end of preceding segment.

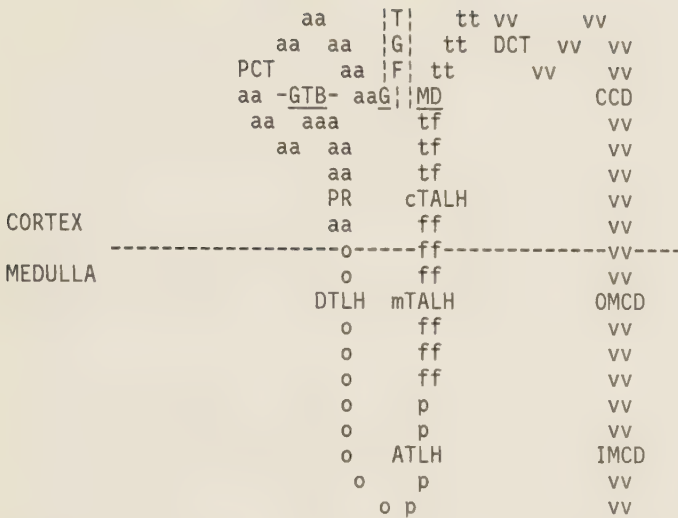


Fig. 1.

Sodium and water reabsorption in different tubular segments.

The nephron consists of several integrated functional units. The first part after the glomerulus (G) is the proximal convoluted tubule (PCT). The loop of Henle includes the pars recta of the proximal tubule (PR), descending thin limb (DTLH), ascending thin limb (ATLH) and thick ascending limb with a medullary part (mTALH) and a cortical part (cTALH) ending in the macula densa (MD) close to the glomerulus. The distal convoluted tubule (DCT) is changed to a collecting duct (CD) after meeting another DCT. The CD can be separated into a cortical (CCD), an outer medullary (OMCD) and an inner medullary part (IMCD).

The small letters refer to sodium and water reabsorption:

a = sodium is actively and passively reabsorbed with water.

o = no sodium, but water, is reabsorbed by the hyperosmolar medulla.

p = sodium, but no water, is passively reabsorbed.

f and t = water impermeable segments with active sodium reabsorption sensitive to loop diuretics (f; cf. Fig. 2) and/or thiazides (t).

v = aldosterone-dependent active sodium reabsorption and ADH-dependent passive water reabsorption.

The sites of glomerulo-tubular balance (GTB) and tubulo-glomerular feedback (TGF) mechanisms are indicated.

an unknown way, resulting in a decrease in the filtration rate, and consequently in the tubular flow rate and ultimately in the salt concentration at the macula densa.

Interstitial and capillary hydrostatic and oncotic pressure gradients, related to ECV, also influence tubular reabsorption (21, 101) and modulate the tubulo-glomerular feedback control (100).

Hormonal mechanisms involved in ECV regulation can be systemic or intrarenal (local hormones such as prostaglandins and adenosine). ECV contraction (activating vascular and atrial volume receptors) is a strong stimulus of sympathetic nerves and of the renin-angiotensin-aldosterone system (RAAS), while ECV expansion suppresses these systems and induces release of the natriuretic hormone (56, 129) and atrial natriuretic peptides (ANP; 62, 85).

Stimulation of renal sympathetic nerves decreases and redistributes renal blood flow, decreases GFR and increases the proximal tubular reabsorption of sodium (8, 9, 10, 63, 130). Further, it increases renin release (66, 134).

Angiotensin II (AII), the primary effector substance of RAAS, has effects on the renal blood flow, GFR and proximal tubular reabsorption similar to those of sympathetic nerve stimulation and potentiates its effects (69, 88, 136).

Aldosterone is released by AII and this release is opposed by ANP and hypokalaemia. It increases sodium reabsorption and potassium secretion (Na^+/K^+ exchange) in the distal tubule, mainly in the cortical collecting tubule.

ANP were discovered by de Bold et al. in 1981 (34) and these atrial peptides seem to be important in volume homeostasis and blood pressure regulation. In the kidneys they counteract vasoconstrictors, and increase GFR, renal blood flow, diuresis and natriuresis (85, 86), and inhibit RAAS (40). There is evidence of another more long-acting natriuretic factor, the natriuretic hormone or the digitalis-like factor (56, 129), which may be just as important in volume homeostasis as ANP.

The prostaglandins are important in the regulation of renal blood flow, GFR, renin secretion and tubular reabsorption (99, 135). PGE_2 and PGI_2 counteract the renal hypoperfusion caused by sympathetic and RAAS activation (63) and maintain GFR in hypovolaemia and renal disease. Therefore, decreasing the synthesis of prostaglandins by cyclooxygenase inhibitors, e.g. non-steroidal anti-inflammatory drugs (NSAID; 37) may deteriorate renal function and even cause acute renal failure in patients with renal disease or with high circulating levels of vasoconstrictor hormones (27).

Adenosine is important in metabolic control of organ function, probably including renal function, i.e. in the regulation of renal blood flow and sodium transport (97, 114).

Water balance is secondary to sodium balance, which determines the water flow over cell membranes and also influences the water intake and excretion via osmotic stimulation of thirst and secretion of arginine vasopressin (AVP). Besides decreasing renal water excretion, AVP also increases tubular sodium reabsorption, probably by increasing the number of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters (61).

Primary renal effects of loop diuretics

Suki et al. (121) observed that furosemide inhibited both free water clearance and free water reabsorption, indicating an effect in TALH. In micropuncture studies (87) the same site of action was found. In experiments with isolated perfused tubules, Burg et al. (25) noted that furosemide in the lumen but not in the surrounding bath inhibited active chloride transport and abolished the lumen-positive transepithelial voltage. They also found that Na^+/K^+ -ATPase activity in the basolateral membrane was necessary for the chloride transport (Fig. 2).

There is a close relationship between tubular secretion of loop diuretics and their saluretic effect (90, 91). Experiments with probenecid in humans have verified that furosemide (65, 93), bumetanide (95) and piretanide (94) exert their diuretic effects primarily from the luminal side (urine) and not from the peritubular side (blood) of the nephron.

Recently, the molecular mechanism of action of loop diuretics such as furosemide and piretanide has been clarified (Fig. 2; 53, 54). These diuretics specifically inhibit a secondarily active $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter. For the rabbit cortical TALH, Schlatter et al (110) reported an IC_{50} (50 % inhibition) of $3 \cdot 10^{-6}$ M (=1 ug/ml) for furosemide. Corresponding values for bumetanide ($\text{IC}_{50} = 2 \cdot 10^{-7}$ M) and piretanide ($\text{IC}_{50} = 10^{-6}$ M) indicate that these drugs have higher potency on a receptor level and of the same magnitude as has been documented in vivo (16, 84). This cotransporter is located in the luminal membrane of the tubular epithelium, but is also found in several other organs, e.g. the cornea, colon,

pancreas, trachea and red blood cells, probably taking part in transport and volume regulation (44, 64).

There is recent evidence in favour of a regulation of the amount and the affinity of the $\text{Na}^+/\text{K}^+2\text{Cl}^-$ cotransporter in the tubular cells (44, 111); this regulation might alter the sodium chloride reabsorption in TALH and thus be involved in salt and water homeostasis.

The transport properties in TALH have recently been thoroughly reviewed (55, 60, 61).

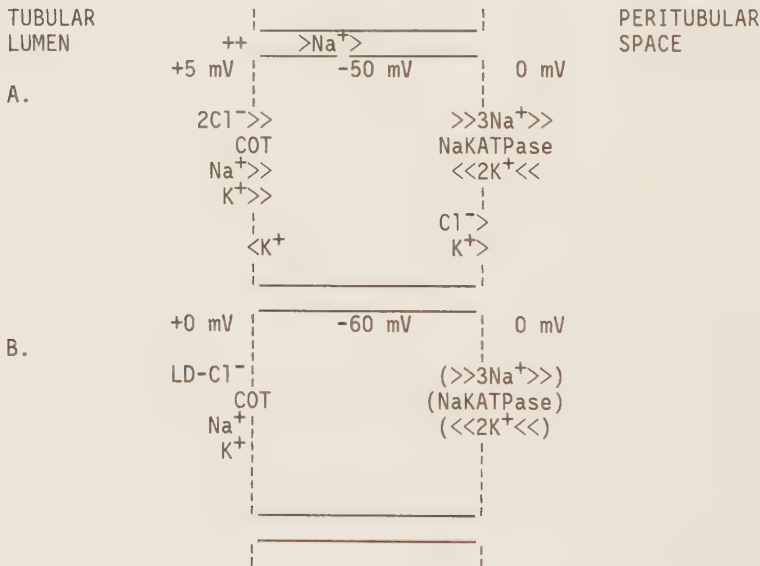


Fig. 2. Model of a tubular cell in the thick ascending limb of Henle's loop (after ref. 54, 79)

Symbols are: >> active or secondary active transport, > diffusive pathway, () reduced effect.

A. Na^+ , K^+ and Cl^- are transported into the cell by the $\text{Na}^+/\text{K}^+2\text{Cl}^-$ cotransporter (COT) and Na^+ is actively pumped out of the cell by the Na^+/K^+ ATPase in the basolateral membrane. K^+ passively diffuses back to the lumen, creating the lumen positive potential, which drives Na^+ out of the lumen by the paracellular shunt pathway. K^+ , like Cl^- , also passively diffuses out of the cell to the peritubular space.

B. Loop diuretics (LD) block one of the Cl^- sites of the COT, which subsequently reduces all the other transports and eliminates the lumen positive potential.

Secondary renal effects of loop diuretics

The primary specific action of loop diuretics - inhibition of the $\text{Na}^+/\text{K}^+2\text{Cl}^-$ cotransport in TALH (Fig. 2) - leads to many secondary effects.

1. The tubulo-glomerular feedback mechanism, which counteracts variations in tubular flow rate by altering GFR, is inhibited by loop diuretics such as furosemide (133) and piretanide (94). This is very important for the diuretic response, which otherwise would be counteracted by a decrease in GFR like that caused by acetazolamide (102).
2. The positive luminal potential in TALH, which is abolished by loop diuretics, is important for cation transport (H^+ , Na^+ , K^+ , $\text{Li}^+(?)$, Ca^{++} , Mg^{++}); 50 % of the sodium absorbed in the TALH passes through the paracellular pathway (60).
3. The sodium chloride transport in TALH and the low water permeability of this segment creates the necessary conditions for medullary interstitial hypertonicity and for the generation of hypotonic tubular urine at the end of TALH. Loop diuretics therefore destroy the ability to concentrate and dilute the urine (121), which becomes iso-osmotic, and increase the tubular flow rate. The latter may influence the tubular reabsorption in other segments of the nephron and even affect the reabsorption of non-electrolytes such as creatinine, urea and uric acid.
4. The increased salt concentration in the distal tubule and collecting duct increases absolute sodium reabsorption and potassium secretion (Na^+/K^+ exchange) in these segments.
5. Loop diuretics block one way of sodium entry to the tubular cells, thus dramatically reducing the $\text{Na}^+/\text{K}^+\text{ATPase}$ activity and the oxygen consumption (54), which means that these drugs can prevent ischaemic damage to the TALH (55).
6. Furosemide induces renin release (72) via several possible mechanisms: 1) sympathetic nerve activation (66, 134), 2) more direct renal effects on vessels and intrarenal baroreceptors (31), or 3) on

the macula densa (127), or 4) via the prostaglandin system (33, 82). These possible mechanisms of renin release may be important with respect to tolerance and rebound phenomena.

7. Furosemide may interact with other transport proteins and enzymes such as a) carbonic anhydrase (105) and b) prostaglandin dehydrogenase (1).

a) The possible inhibitory effect of furosemide on carbonic anhydrase, which would decrease proximal tubular reabsorption and alkalinize the urine, is controversial (26, 105, 126) and probably not important in clinical situations except perhaps in high-dose treatment in uraemic patients.

b) Inhibition of prostaglandin dehydrogenase will increase the intrarenal concentration of prostaglandins, which may enhance renal blood flow and renin secretion (33, 82, 98). Prostaglandins released from the kidney may also decrease the peripheral vascular resistance (14).

Delivery of a loop diuretic to its site of action

The diuretic effect of a loop diuretic is directly related to the rate of drug excreted in the urine (25, 65, 93).

The pharmacokinetic determinants of delivery of a loop diuretic to its site of action in TALH are its bioavailability, renal clearance, non-renal clearance, volume of distribution, plasma concentration, and protein binding in the plasma and urine. These variables are to a large extent interdependent and change with the type and severity of many diseases.

The bioavailability of furosemide is low and variable (11). In one study after food intake it ranged from 27 to 80 %, with a mean of 43 % (59) and in another it was 17 % in an oedematous and diuretic resistant state as compared to 75 % in an oedema-free and responsive state (92). It has been suggested that the bioavailability of piretanide is high and less variable (18).

Some pharmacokinetic parameters of furosemide are shown in Table II. The renal clearance of piretanide is similar and averages 100 ml/min, but its non-renal clearance, which is similar to its renal clearance, is somewhat higher than that for furosemide (7, 18, 84).

The binding of piretanide to plasma albumin is about 95 % (39) and that of furosemide even higher (Table II). The fraction of the drug in plasma filtered at the glomeruli is therefore very small and the major part of its renal clearance is due to active tubular secretion by the organic acid transport system (93, 94).

The fraction of a given dose excreted unchanged in the urine varies (65, 83, 117). Besides being dependent on the bioavailability of an oral dose, this fraction may be influenced by both renal and liver disease. The time course of the urinary delivery is also important for the total effect (58, 71).

Table II

Furosemide pharmacokinetics in normal volunteers following intravenous administration.

Reference	N	$t_{1/2}$ (min)	Cl_r (ml/min)	Cl_{nr} (ml/min)	V_d (ml/kg)	uf (%)
Allgulander et al. 1980 (3)	5	52 (15)	95 (24)	99 (48)	210 (56)	1.9 (0.2)
Smith et al. 1980 (117)	9	92 (7)	110 (24)	54 (10)	109 (19)	1.2 (0.2)
Andreassen et al. 1982 (4)	10	70 (21)	114 (33)	56 (19)	132 (22)	1.4 (0.4)
Hammarlund et al. 1984 (59)	8	51 (4)	117 (32)	45 (12)	129 (23)	

Abbreviations: N = number of subjects; $t_{1/2}$ = elimination half-life; Cl_r = renal clearance; Cl_{nr} = non-renal clearance; V_d = volume of distribution; uf = unbound fraction.
S.D. in brackets.

Renal sensitivity to loop diuretics

The renal sensitivity to a loop diuretic is characterized by the relationship between the urinary drug excretion rate and the diuretic effect, which in healthy volunteers gives a sigmoid-shaped curve (28).

Loop diuretics exert their action at one intermediary segment of the nephron, TALH. Thus, modifications of the total diuretic response can take place at both proximal and distal tubular segments, mediated by normal (tolerance) or pathophysiological compensatory mechanisms (resistance).

The occurrence of a decrease in the natriuretic effect of a diuretic after prolonged administration is well documented (6, 43, 51). Grantham called this the "braking phenomenon" and it has been proposed that it may result from increased sodium reabsorption in the proximal and distal tubules secondary to extracellular volume contraction. Thus, important determinants of the diuretic response are the water and electrolyte status and ECV (17, 38, 78, 131).

Recently it has been shown that the renal sensitivity to furosemide decreased with time after a single oral dose, a process referred to as "acute tolerance" development (58).

The physiological mechanisms behind the "braking phenomenon" and "acute tolerance" to loop diuretics may also contribute to the impaired diuretic effect (diuretic resistance) seen in patients.

AIM OF THE STUDY

The principal aim of this study was to address certain questions regarding the mechanisms of tolerance and resistance to the renal effects of loop diuretics and some methodological aspects of investigations with diuretics:

The specific aims were:

1. to investigate renal handling and effects of furosemide in healthy subjects during controlled isotonic dehydration and after complete and continuous restoration of volume losses (I:A and B);
2. to assess a) how the renal response and the intrinsic sensitivity to furosemide in healthy volunteers change during treatment for one week and b) whether inhibition of ACE achieved by concomitant treatment with lisinopril would affect this sensitivity (II);
- 3
 - a. to study the dose-response relationship and the safety of piretanide in dialysis patients,
 - b. to compare the pharmacokinetics and effects of piretanide in predialysis renal patients (GFR 7-28 ml/min) with those seen in healthy volunteers, and
 - c. to determine the urinary excretion-effect relationship of piretanide in patients with renal failure (dialysis and predialysis) and in healthy volunteers (III);
4. to investigate how acute changes in plasma and urinary albumin concentrations caused by two different modes of plasma volume expansion (albumin and dextran infusions) would affect renal salt excretion and the pharmacokinetics and pharmacodynamics of furosemide in nephrotic patients (IV);
5. to evaluate the reliability of lithium as a marker of proximal tubular reabsorption of sodium (V); and
6. to evaluate the reliability of creatinine as a marker for GFR in different stages of hydration in healthy volunteers during large variations in urinary flow rates (VI).

MATERIALS AND METHODS

The studies were approved by the local Ethics Committees.

Subjects

Healthy volunteers: Eight healthy men (aged 30 - 43 years) participated in the study on acute tolerance (I, V and VI), 12 men (aged 22 - 39 years) in the study on delayed tolerance (II) and six men and women (aged 23 - 34 years) volunteered as controls in the piretanide study (III).

Patients (Table III): Twenty-two patients with chronic renal failure participated in the study on pharmacokinetics and pharmacodynamics of piretanide (III) and five nephrotic patients participated in the study on effects of plasma volume expansion on furosemide pharmacokinetics and pharmacodynamics (IV).

Table III

Characteristics of patients studied.
Means $\pm$ S.D. with range in brackets.

	<u>Predialysis pts</u>	<u>Dialysis pts</u>	<u>Nephrotic pts</u>
Males	7	6	4
Females	5	4	1
Age (yrs)	54 $\pm$ 14	47 $\pm$ 13	48 $\pm$ 14
Range	(21 - 65)	(21 - 61)	(33 - 60)
Cl _{EDTA or inulin} ml/min/1.73 m ²	17 $\pm$ 7 (7 - 28)		75 $\pm$ 34 (27 - 110)
Cl _{creatinine} ml/min/1.73 m ²	21 $\pm$ 11 (6 - 37)	4 $\pm$ 2 (1 - 7)	109 $\pm$ 49 (49 - 163)
Proteinuria g/24 h			8.8 $\pm$ 5.0 (4.4 - 16.9)

Experimental procedures

Studies I, V and VI: After a control period (PC) of 1 h, eight healthy men were given 10 mg furosemide i.v. (Impugan, Dumex Ltd, Denmark) followed by a continuous i.v. infusion of 8 mg/h (Fig. 3). After 2 h of dehydration (P3 - P6) the subjects were rapidly rehydrated (P7 - P8) and kept in isovolaemia by water given orally and intravenous saline (P9 - P12). Clearance values were determined during 30-min periods and venous blood samples were drawn in the middle of each period. Six mMol lithium citrate had been taken 10 and 2 h before start.

Inulin													
PAH	Bolus + infusion												
	Rehydration												
	Furosemide bolus-infusion												
	-1	0	1	2	3	4	5	6 h.					
		PC	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	
Dehydrated state (DS)													
Rehydrated state (RS)													
Urine													
Blood/serum		B	B	B	B	B		B	B	B	B	B	
Plasma		P				P						P	
Body weight	W			W		W		W		W		W	
BP/PR	BP			BP		BP		BP		BP		BP	
Conjunctival pO ₂	C					C		C					

Analyses:

Serum and urine: sodium, potassium, lithium, chloride, osmolality, albumin, creatinine, urea, inulin, PAH, furosemide.

Plasma: norepinephrine, epinephrine, dopamine, renin activity, aldosterone, arginine vasopressin, atrial natriuretic peptides.

* BP/PR = Blood pressure and pulse rate.

Fig. 3. Design of the acute tolerance study (I, V, VI)

Study II: The design was that of an open, randomized, multiple dose, three-period, cross-over study. Each subject received each of the following treatments:

L protocol: lisinopril (MK-521; Merck Sharp & Dohme, Rahway, NJ, USA) 20 mg orally daily for 7 days and placebo i.v. on day 1 and day 7;

F protocol: furosemide (ACO läkemedel AB, Solna, Sweden) 40 mg daily for 7 days, i.v. day 1 and day 7, orally the other days.

L+F protocol: lisinopril 20 mg orally daily for 7 days and furosemide 40 mg daily for 7 days, i.v. on day 1 and on day 7, orally on the other days.

On days 1 and 7 the urinary excretion rates of drugs and electrolytes were determined. On day 7 blood samples were also drawn to get plasma levels of the drugs and clearance values of furosemide, inulin and PAH. Albumin, the haematocrit and aldosterone were determined 0 and 5.5 h after administration of drugs on day 7 (Fig. 4).

<u>Inulin, PAH; Bolus + infusion</u>													
<u>Lisinopril 20 mg orally (L)</u>													
<u>Furosemide 40 mg i.v. (F)</u>													
<u>L+F</u>													
	-1	0	1	2	3	4	5	6	8	10	12	24 h	
<u>Urine</u>													
<u>S-F</u>		SSSSS	S	S	S	S	S		S	S	S		S
<u>S-L</u>		S S S	S	S	S	S	S		S	S	S		S
<u>S-Inul., PAH</u>		S	S	S	S	S	S						
<u>Haematocrit</u>	H							H					
<u>S-Albumin</u>	S							S					
<u>Aldosterone</u>	S							S					
<u>Body weight</u>	W							W					
<u>BP/PR*</u>	BP							BP			BP		

Urine analyses: Sodium, potassium, magnesium, chloride, inulin, PAH, furosemide (F), lisinopril (L).

* BP/PR = Blood pressure and pulse rate.

Fig. 4. Design of the delayed tolerance study, day 7 (II).

Study III: A. The dialysis patients (DP) participated in an oral single-dose seven-step study, in which placebo, piretanide 24, 48, 72 and 96 mg, placebo and furosemide 750 mg were administered in the morning on a day immediately preceding a day of dialysis. The same day of the week was used for the test. Urine was collected during the periods 0-6 and 6-24 h after drug administration.

B. The predialysis patients (PDP) participated in three experiments, when they were given placebo, piretanide i.v. and piretanide orally (Fig. 5). After the control day, the patients were given test doses of piretanide orally, starting at 24 mg and increasing every second day first to 48 mg and then to 96 mg up to the dose which increased the diuresis during a 10-h period by at least 50 % above the control value. Two weeks after the control day the dose of piretanide which fulfilled this criterion was given i.v. at an infusion rate of about 10 mg/min, and one week later the same dose were given orally. Blood was drawn and urine collected according to the scheme of the study (Fig. 5).

C. The healthy volunteers (HV) participated in two experiments, in which they were given 6 mg of piretanide and placebo i.v. in randomized order. Blood and urine samples were taken according to the same scheme as for the predialysis patients (Fig. 5).

	Placebo or Piretanide orally or i.v.											
	0	1	2	3	4	5	6	7	8	10		24 h
Urine												
S-piretanide	S	S	S	S	S	S	S	S	S	S		S
Serum	S											S
Audiometry	A	A										A

Serum and urine analyses: albumin, sodium, potassium, calcium, magnesium, chloride, phosphate, osmolality, creatinine, urea, urate, piretanide.

Fig. 5. Design of the piretanide study (III).

Study IV: Five patients with nephrotic syndrome in a stable clinical condition participated in a three-step study (Fig. 6) with a control period with furosemide (day 1, C+F protocol) and two study periods with two different modes of plasma volume expansion and furosemide (days 2 and 5; A+F or D+F protocol). During study days A+F and D+F 40 g of albumin (KabiVitrum AB, Sweden) or 36 g of Dextran 70 (Macrodex, Pharmacia AB, Sweden) was given at 0.5-1.5 h in a 600 ml solution, which also contained 92 mmol sodium chloride. The concentrations were chosen to result in a similar volume expansion with no changes in plasma sodium levels.

At 4 h 40 mg of furosemide (Impugan, Dumex Ltd, Denmark) was administered i.v.. Urinary output was replaced by i.v. saline and water orally.

Blood and urine samples were taken for studies of furosemide pharmacokinetics, clearance values and the urinary excretion rate of electrolytes. Plasma ANP and aldosterone were determined at 4 h (before furosemide) and at 5 and 7 h.

<u>Inulin, PAH; Bolus + infusion</u>												
<u>Protocol</u>	C+F Albumin A+F Dextran D+F infusion (0.5-1.5 h)											
	Furosemide 40 mg i.v. (F)											
	0	2	4	4.5	5	5.5	6	7	8	10	24 h	
<u>Urine</u>												
<u>Serum</u>	S		S		S			S				S
<u>Hct</u>	H		H		H			H				H
<u>Plasma</u>	P		P		P			P				
<u>S-F</u>			S S S S S		S		S	S		S		S
<u>S-Inul., PAH</u>	S		S		S		S	S				
<u>Weight</u>	W		W									W
<u>BP/PR*</u>	BP		BP									BP

Urine and serum analyses: Sodium, potassium, chloride, albumin, IgG, creatinine, urea, inulin, PAH and furosemide.

Plasma analyses: Atrial natriuretic peptides and aldosterone.

* BP/PR = Blood pressure and pulse rate.

Fig. 6. Design of the study on nephrotic patients (IV).

Methods:

Monitoring of peripheral circulation (I): Conjunctival oxygen tension was measured (Conjunctival oxygen sensor, Orange Medical Instruments Inc., Costa Mesa, Calif., USA) in periods PC, P6 and P8 according to the manufacturer's directions for use.

Neurohormonal analysis: Plasma norepinephrine, epinephrine and dopamine (I) were assayed by high-performance liquid chromatography. Plasma renin activity (PRA; I and IV), plasma aldosterone (I, II and IV), plasma ANP (I, IV) and arginine vasopressin (I) were measured by radioimmunoassay.

Chemical analyses: Concentrations of furosemide and piretanide in plasma and urine [I, II, III (predialysis patients) and IV] were measured by high performance liquid chromatography. The concentrations of piretanide in plasma and urine from the healthy volunteers and dialysis patients in study III, and of lisinopril in study II, were determined by radioimmunoassay. Colorimetric methods were used for measurements of inulin and PAH (I, II and IV).

Haemoglobin and the haematocrit in blood, the concentrations of sodium, potassium, chloride, albumin and creatinine in serum and urine, and the osmolality of serum and urine were determined according to routine methods of the hospital. Lithium in plasma and urine (V) was measured by atomic absorption photometry.

Calculations: The apparent volume of distribution, all clearance values (Cl) and all urinary excretions of water and electrolytes and proteins and drugs were normalized to a body surface area of 1.73 m^2 .

Mean arterial blood pressure (MBP) was calculated as diastolic blood pressure + $1/3 \times$ pulse pressure. Renal blood flow (RBF) was calculated as $\text{Cl}_{\text{PAH}} / (1 - \text{haematocrit})$ and renal vascular resistance as MBP / RBF ($\text{mmHg} \times \text{min} / \text{l} = 80 \times \text{dynes} \times \text{s} / \text{cm}^5$), neglecting renal venous pressure.

Plasma volume (PV) was determined in the nephrosis study (IV), before the start of the albumin or dextran infusion, as the volume of distribution of the administered dose of ^{125}I -albumin.

Blood volume (BV) was calculated as $PV/(1 - 0.9 \times H)$. The figure 0.9 represents a mean of the ratio of whole body haematocrit to venous haematocrit (H; 46). Red cell volume (RCV) is $0.9 \times H \times BV$. It was continuously corrected for losses of erythrocytes due to the blood sampling, but otherwise assumed to be constant. Changes in blood and plasma volumes were estimated from the currently corrected RCV and haematocrit (IV).

In the acute tolerance study (I), plasma volume was not determined but was estimated as 4.4 % of the total body weight (46) and changes in the haematocrit and serum albumin were used to estimate the changes in blood and plasma volumes. Corrections were made for losses of erythrocytes and albumin due to the extensive blood sampling (a total of 500 ml during the study day).

Pharmacokinetic analysis: The plasma kinetics of piretanide (III) and furosemide (II and IV) was analysed by means of a two-compartment open model. Disposition rate constants (α and β) were calculated by linear regression analysis after logarithmic transformation. The area under the plasma concentration time curve (AUC) was estimated with use of the trapezoidal rule and extrapolated to infinity (III).

Bioavailability (f) was calculated as

$$f = \text{AUC orally} / \text{AUC i.v.} \quad (\text{eq. 1})$$

The plasma elimination half-life ($t_{1/2}$) was estimated as

$$t_{1/2} = \ln 2 / \alpha \text{ or } \beta \quad (\text{eq. 2})$$

Apparent volume of distribution (V_d) was calculated as

$$V_d = \text{Dose i.v.} / (\text{AUC i.v.} \times \beta), \quad (\text{eq. 3})$$

plasma clearance (Cl_p) as

$$Cl_p = \text{Dose i.v.} / \text{AUC i.v.} \quad (\text{eq. 4})$$

and renal clearance (Cl_r) as

$$Cl_r = \text{Urinary recovery of drug} / \text{AUC} \quad (\text{eq. 5})$$

Non-renal clearance (Cl_{nr}) was calculated as

$$Cl_{nr} = Cl_p - Cl_r \quad (\text{eq. 6})$$

In studies I, II and IV the following equations were used:

$$Cl_r = \text{urinary excretion rate} / P_f \quad (\text{eq. 7}),$$

where P_f is the plasma furosemide concentration in the middle of the clearance period.

$$Cl_p = Cl_r/f_e, \quad (\text{eq. 8}),$$

where f_e is the fraction of the given dose excreted unchanged in the urine.

$$V_d = Cl_p/\beta. \quad (\text{eq. 9})$$

Statistical analysis: Standard statistical methods were used throughout. Means and standard deviation (S.D.) were computed conventionally, using $(n-1)$ as a divisor in the S.D. Results are given as arithmetic means $\pm$ S.D. Correlation coefficients were the ordinary product-moment coefficients. Differences between different treatments and between corresponding periods on day 1 and day 7 in the delayed tolerance study (II) were analysed by the t test for paired samples and were considered significant at the 0.05 level.

RESULTS

Acute tolerance to furosemide (Study IA and B)

The excretion of water, sodium and chloride increased substantially during the two periods after the bolus injection and the start of the infusion of furosemide (Fig. 7). Thereafter the diuretic and saluretic responses decreased during dehydration. After rehydration with saline the diuresis and saluresis increased to levels even higher than before dehydration.

During dehydration the subjects lost 1.8 ± 0.3 kg in body weight. The plasma volume decreased by $14 \pm 4\%$ and the blood volume by $9 \pm 3\%$. Rehydration rapidly restored the body weight and the plasma and blood volumes to control values. There were no significant changes in supine blood pressure or heart rate.

The reduction in the diuretic effect during dehydration was accompanied by significantly increased plasma levels of norepinephrine (1.38 to 2.14 nmol/l), PRA (0.52 to 1.13 ng/ml/hr) and aldosterone (0.29 to 0.45 nmol/l), all of which then decreased after rehydration. There were no significant changes in AVP.

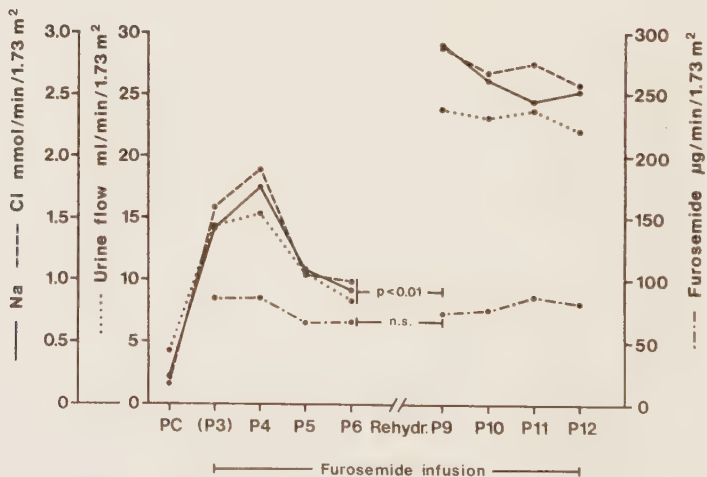


Fig. 7

Effect of dehydration (P3 - P6) and isovolaemia after rapid rehydration (P9 - P12) on furosemide-induced diuresis and on the urinary sodium, chloride and furosemide excretion rates. Furosemide infusion was started in P3. (From I).

There was a marked decrease in conjunctival pO_2 during dehydration, indicating decreased peripheral circulation, from 58 ± 7 mm Hg (PC) to 42 ± 5 mm Hg (P6; $p < 0.001$), followed by an increase to 57 ± 4 mm Hg (P8) after rehydration.

The renal sensitivity to furosemide decreased during dehydration and increased again after rehydration (Figs 21 and 22).

The change in the renal clearance of furosemide from the dehydrated state (DS; P5-P6) to the rehydrated state (RS; P9-P12) followed the same pattern as that of inulin and PAH. The urinary furosemide excretion rate ($U_F \cdot V$) was less influenced (Table IV and Fig. 7).

Table IV: Means and S.D. of urine flow, clearance values (Cl), and plasma (P) and urinary (U) concentrations of furosemide (F) and albumin during the dehydrated state (DS) and rehydrated state (RS).

	DS		RS	
Urine flow (V)	9.5 (1.8)	***	23.2 (5.1)	ml/min * 1.73 m ²
Cl _{Inulin}	92 (14)	***	109 (14)	"
Cl _{PAH}	402 (160)	**	622 (199)	"
Cl _{F, renal}	71 (17)	***	109 (27)	"
Cl _{F, non-renal}	65 (15)	n.s.	65 (21)	"
$U_F \cdot V$	66 (11)	**	79 (15)	ug/min * 1.73 m ²
P _F	1.0 (0.2)	***	0.8 (0.2)	ug/ml
U _F	7.3 (1.9)	***	3.5 (0.2)	ug/ml
P _{albumin}	48.2 (1.4)	***	40.4 (1.5)	g/l

n.s. = non-significant, ** = $p < 0.01$, *** = $p < 0.001$.

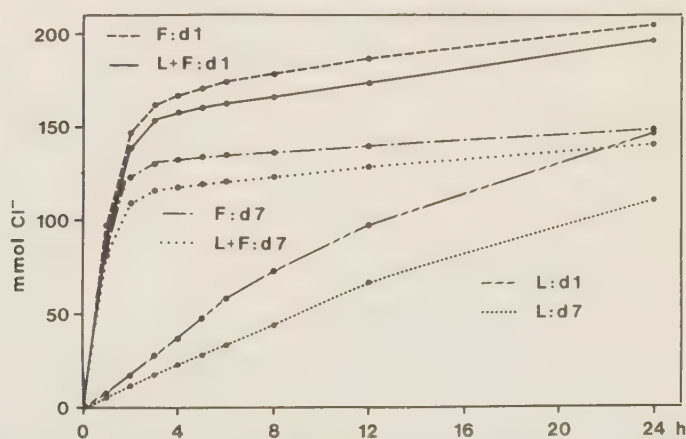


Fig. 8. Cumulated urinary excretion of chloride after a dose during days 1 and 7 of each protocol in the delayed tolerance study (II). The excretion rate is indicated by the slope of the curve. The flattening of the F and L+F curves after 4 h shows "rebound effects" and the decrease of the same curves on day 7 indicates the "delayed tolerance".

Delayed tolerance to furosemide (Study II)

A daily dose of 40 mg of furosemide for one week had a brisk, acute diuretic effect, but did not lead to dehydration (unchanged body weight), intravascular hypovolaemia (unchanged haematocrit and plasma albumin), hyponatraemia or a fall in blood pressure. There was a decrease in the response to furosemide from day 1 to day 7 and angiotensin converting enzyme (ACE) inhibition with lisinopril 20 mg daily did not alter the response (Fig. 8). The renal furosemide excretion rate was unchanged on all four days (Fig. 20) of i.v. administration of furosemide and the urinary recovery was $64 \pm 9\%$ of the given dose. Thus, the renal sensitivity to furosemide was reduced on day 7 as compared to day 1 with both the F and L+F protocols (Fig. 9).

Renal clearances are shown in Figure 10. The renal vascular resistance was equal in all protocols on day 7 during the first hour after the intake of the drug(s) but only in the F protocol was it significantly increased 5-6 h after the dose.

On day 7 the plasma aldosterone values in the L and L+F protocols decreased significantly from 0 to 5.5 h. At 5.5 h they were significantly lower than in the F protocol.

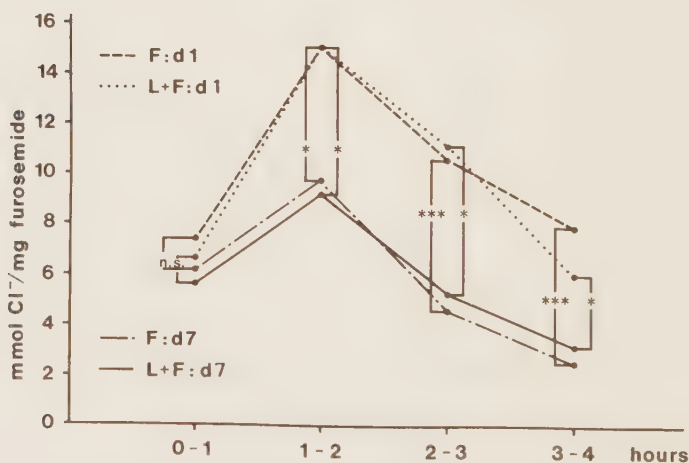


Fig. 9

Efficiency of furosemide in the delayed tolerance study (II) during the first 4 h after an i.v. dose with the F and L+F protocols.

Significant differences between day 1 and day 7 are indicated: n.s. = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

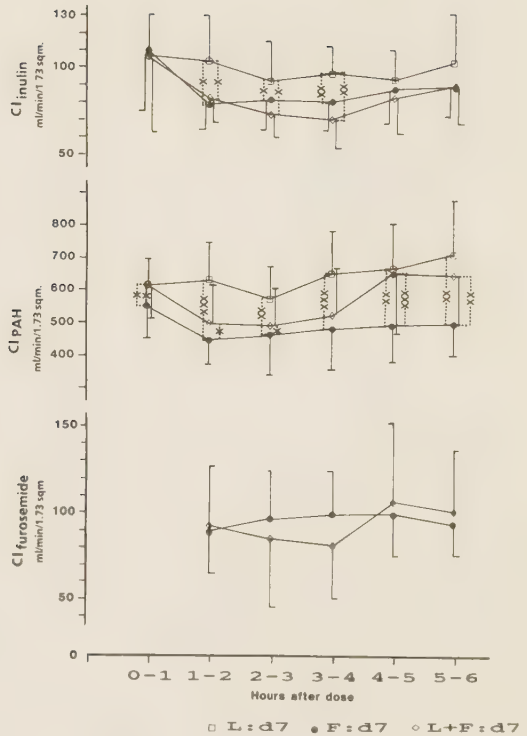


Fig. 10

Means $\pm$ S.D. of renal clearance values for inulin, PAH and furosemide on day 7, during the first 6 h after the dose. Delayed tolerance study (II), protocols L, F, L+F. Significant differences are indicated: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

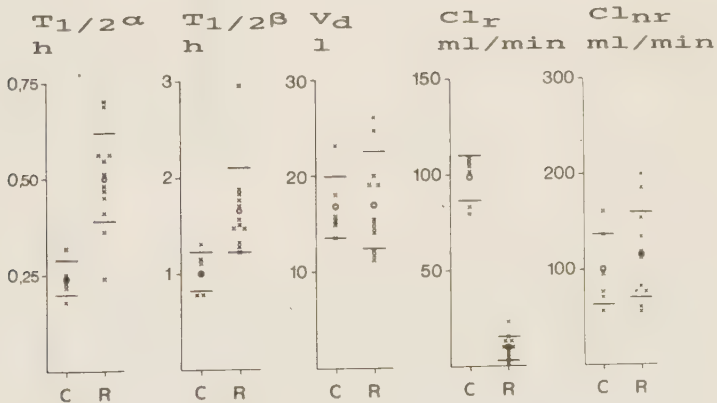


Fig. 11

Pharmacokinetic parameters of piretanide in healthy volunteers (C) and patients with chronic renal failure (GFR = 7-28 ml/min) (R). Individual data (x), means (o) and S.D. (—).

Piretanide effects in healthy and uraemic subjects (Study III)

In the predialysis patients the pharmacokinetic parameters of piretanide (Fig. 11) showed considerably decreased renal clearance but the non-renal clearance and V_d were unchanged. This resulted in a prolonged elimination half-life (1.66 ± 0.44 h). The bioavailability in the same group was $92 \pm 15\%$.

In the healthy volunteers 6 mg of piretanide had a substantial saluretic effect which lasted for 3 h. After 6 h the saluresis was significantly lower than during the control period (Fig. 12).

In the predialysis patients the response curve for the i.v. dose was lower and flatter than in the healthy volunteers and it did not fall below the control values until after 10 h (Fig. 12). The oral dose gave a later response, but the 24 h effect was the same.

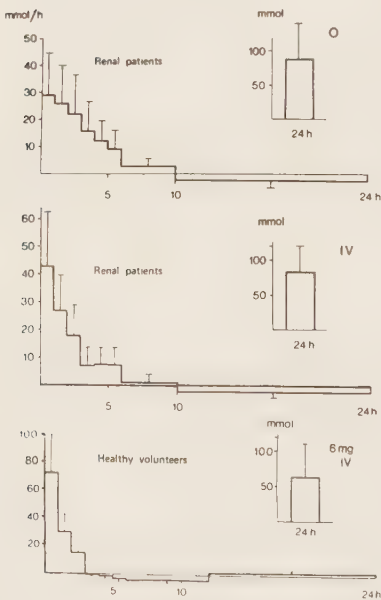


Fig. 12. Increase in urinary excretion of chloride after administration of piretanide i.v. and orally to predialysis patients and i.v. to healthy volunteers.

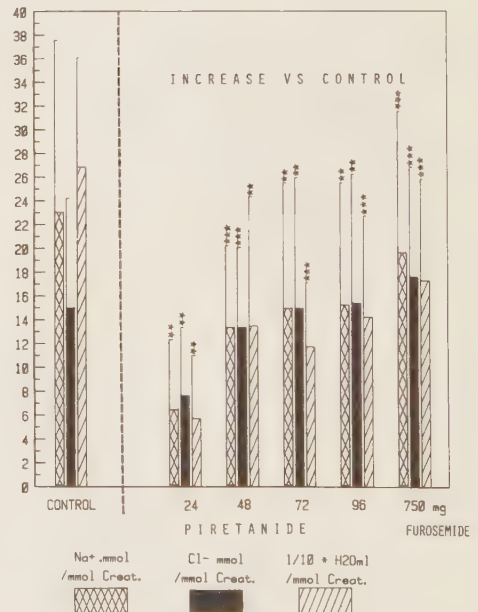


Fig. 13. Control and increase in sodium, chloride and water excretion 6 h after administration of 24 - 96 mg piretanide and 750 mg furosemide in 10 dialysis patients. (From III)

In the dialysis patients piretanide gave a dose-dependent increase in the 6 h diuretic and saluretic response curve (Fig. 13), which flattened after the 48 mg dose. In contrast to the response to furosemide no effect remained after 6 h, but nor were there any rebound effects. The prolonged furosemide effect resulted in an approximately 50 % higher 24 h diuretic effect than after the highest piretanide dose.

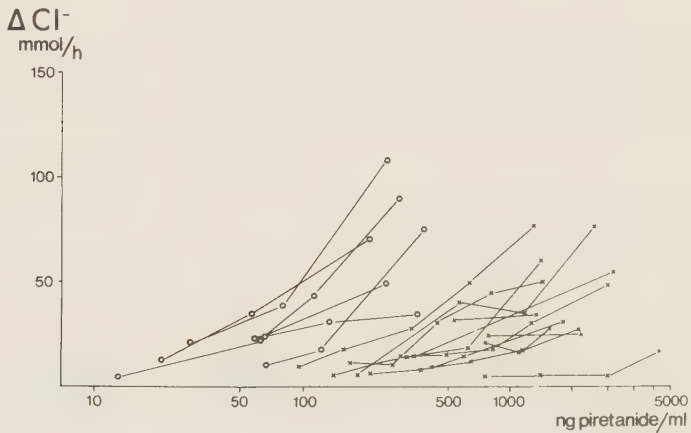


Fig. 14.

Increase in urine chloride excretion related to the plasma piretanide level in healthy volunteers (o) and predialysis patients (x). (From III)

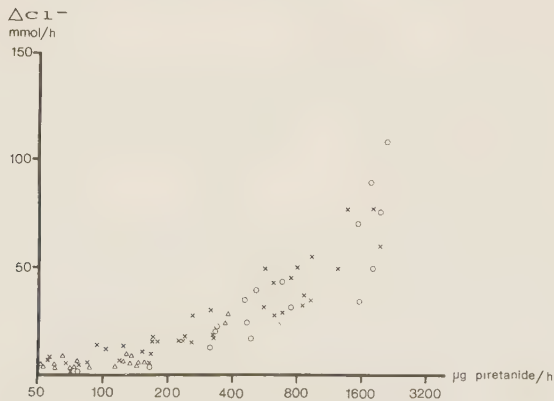


Fig. 15.

Increase in urine chloride excretion related to the urinary piretanide excretion rate in healthy volunteers (o), predialysis patients (x) and dialysis patients (Δ). (From III)

As seen in Figure 14, about ten times higher plasma levels of piretanide were necessary to obtain a certain chloruretic response in the patients than in the healthy volunteers. However, the relationship between the urinary excretion rate of piretanide and its chloruretic effect was similar in healthy volunteers and predialysis and dialysis patients (Fig. 15). When the average piretanide excretion was compared, it was found that the volunteers excreted 49% of the dose in the urine, the predialysis patients 7.3 % and the dialysis patients 0.8 %, which implied an increase in the chloride excretion from 0-6 h of 37, 40, and 37.5 mmol per mg piretanide excreted in the urine, respectively.

Furosemide effects in relation to plasma volume expansion in nephrosis (Study IV)

The initial plasma volume was low in one patient and normal in the others. PRA and the plasma aldosterone concentration were high only in the hypovolaemic patient. The plasma volume expansion amounted to about 20 % and uniformly decreased the aldosterone levels. The initial ANP values were normal, and they increased after dextran but not after albumin infusion. The mean plasma albumin concentration was stable (28 g/l) during the control day but decreased after dextran infusion to 23 g/l and increased after albumin infusion to 33 g/l. The mean urinary albumin excretion rate was 3.9 g/24 h with the C+F and D+F protocols, but increased to 6.6 g/24 h with the A+F protocol. The IgG excretion was unchanged.

The urine flow almost doubled after both modes of plasma volume expansion, as compared with the control period, but the urinary sodium excretion was very little influenced (Table V). Furosemide, on the other hand, increased the sodium excretion by about 20 % (40 mmol) after both modes of plasma volume expansion. However, since the patients had received 92 mmol of sodium chloride together with dextran or albumin, their salt balance was still positive (mean retention 0-8 h: control day +14; dextran day +65; albumin day +83 mmol Na).

Plasma volume expansion induced only minor changes in Cl_{inulin} , but caused a marked increase in Cl_{PAH} after dextran infusion and a small increase after albumin infusion. This resulted in a decrease in the filtration fraction, which was also low on the control day (Table V).

TABLE V:

a. Effects of plasma volume expansion with dextran (D) and albumin (A) compared with control values (C).

Mean values of a 2 h period.

b. Effects of furosemide 40 mg i.v. after plasma volume expansion with dextran (D+F) or albumin (A+F) compared with the control (C+F). Mean values of a 4 h period.

a.	V ml/min			U _{Na} V umol/min			U _{alb} V mg/min		
	C	D	A	C	D	A	C	D	A
Mean	1.6 *	3.1	3.0	152	193	154	2.5	3.5	4.0
S.D.	1.0	1.9	2.1	105	129	91	1.0	1.4	1.8

b.	C+F	D+F	A+F	C+F	D+F	A+F	C+F	D+F	A+F
Mean	7.0	7.1	7.5	589	716	669	3.7	3.8**	5.7
S.D.	4.2	3.3	3.5	356	299	282	1.8	2.1	2.8

a.	Cl _{in} ml/min			Cl _{PAH} ml/min			FF %		
	C	D	A	C	D	A	C	D	A
Mean	78	81	73	528	735	592	19	16	15
S.D.	44	48	43	369	564	401	10	8	6

b.	C+F	D+F	A+F	C+F	D+F	A+F	C+F	D+F	A+F
Mean	74	76	69	504 *	700	532	16	13	14
S.D.	30	23	32	273	350	240	5	5	4

* significant difference, $p < 0.05$, ** = $p < 0.01$.

V = urine flow; U_{Na}V = sodium excretion rate; U_{alb}V = albumin excretion rate; Cl_{in} = inulin clearance; Cl_{PAH} = PAH clearance; FF = filtration fraction.

The renal and non-renal clearance of furosemide (Fig. 16A) and its apparent volume of distribution (Fig. 16B) increased on the day of dextran infusion and decreased on the day of infusion of albumin, effects which were the reverse of the changes in the serum albumin concentration. In contrast, the urinary excretion of furosemide was highest after albumin infusion, 20.4 mg compared with 17.6 mg ($p < 0.001$), during the control day.

There was a tendency towards an increase in the intrinsic renal sensitivity to furosemide following volume expansion (Fig. 17).

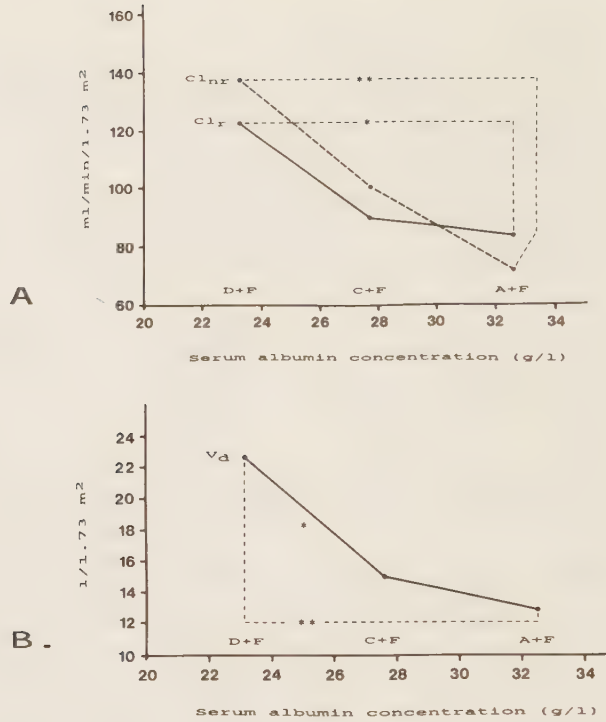


Fig. 16:

Relations between plasma albumin concentration and A) renal (Cl_{r}) and non-renal (Cl_{nr}) clearance of furosemide and B) volume of distribution (V_d).

Significant differences are indicated: * = $p < 0.05$, ** = $p < 0.01$.

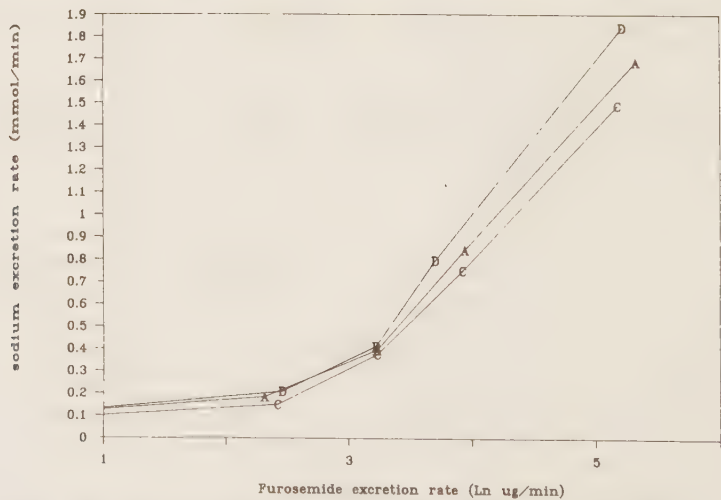


Fig. 17.

Renal sensitivity to furosemide in nephrotic patients under basal conditions (C) and after plasma volume expansion (about 20 %) with dextran (D) and albumin (A).

TABLE VI

Furosemide and piretanide pharmacokinetics.

Study: protocol	N	P_{alb} (g/l)	$t_{1/2}$ (h)	Cl_r (ml/min)	Cl_{nr} (ml/min)	V_d (l/)
Furosemide:						
I:DS	8	48.2 (1.4)		71 (17)	65 (15)	
I:RS	8	40.4 (1.5)		109 (27)	65 (21)	
II:F	12	46.0 (3.0)	1.26 (0.10)	95 (19)	50 (24)	15.7 (3.0)
II:L+F	12	45.8 (2.2)	1.25 (0.09)	93 (27)	50 (13)	15.5 (4.2)
IV:C+F	5	27.7 (5.2)	0.99 (0.25)	90 (63)	101 (56)	14.8 (7.0)
IV:D+F	5	23.3 (6.8)	1.01 (0.18)	123 (51)	138 (29)	22.6 (7.4)
IV:A+F	5	32.6 (5.4)	0.99 (0.24)	84 (51)	72 (38)	12.5 (5.6)
Piretanide:						
III:HV	6	45.3 (2.8)	1.02 (0.20)	98 (12)	100 (37)	16.8 (3.2)
III:PDP	12	39.2 (2.1)	1.66 (0.44)	9 (6)	114 (45)	16.9 (4.7)

Abbreviations: Study protocols: see experimental procedures, pp 19-22.

N = number of subjects; P_{alb} = plasma albumin concentration; $t_{1/2}$ = elimination half-life; Cl_r = renal clearance; Cl_{nr} = non-renal clearance; V_d = volume of distribution.

S.D. in parentheses.

Table VII

Means and S.D. of excretion fractions (EF) of Li^+ , Na^+ , creatinine, urea and EF_{Na^+}/EF_{Li^+} (Study V).

	Furosemide infusion-----			
	Control	Initial Period	After Dehydration	After Rehydration
	CP	P4	P5-6	P10-12
EF_{Li^+} (%)	30 (8)n.s.	40 (8)***	35 (8)	50 (13)**
EF_{Na^+} (%)	1.5 (0.4)***	13.4 (3.5)***	8.1 (2.1)	17.5 (4.7)**
EF_{Na^+}/EF_{Li^+} (%)	5 (2)***	34 (9)**	24 (11)	36 (7)***
EF_{urea} (%)	67 (12)n.s.	63 (9)**	58 (11)	72 (11)*
$EF_{creat.}$ (%)	124 (21)n.s.	134 (35)*	105 (28)	147 (21)**

n.s.=non-significant, *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$.

Lithium clearance as a marker of proximal tubular reabsorption (Study V)

The excretion fraction of lithium (Cl_{Li^+}/Cl_{inulin} , EF_{Li^+}) started at a higher level than EF_{Na^+} but changed after administration of furosemide in the same way as the latter variable (Table VII). Initially EF_{urea} changed in an opposite way to the others, but subsequently it followed their pattern. The proportional changes in EF, relative to the control period (CP) were much smaller for Li^+ and urea, however, than for Na^+ .

Creatinine clearance during furosemide diuresis (Study VI)

The clearance values of inulin, PAH, creatinine and urea decreased during dehydration and increased significantly from the dehydrated to the rehydrated state (Fig. 18). There were even significant changes in the excretion fractions of creatinine and urea (Table VII).

EF_{creat} changed considerably from 1.05 in the dehydrated state to 1.47 in the state of rehydration.

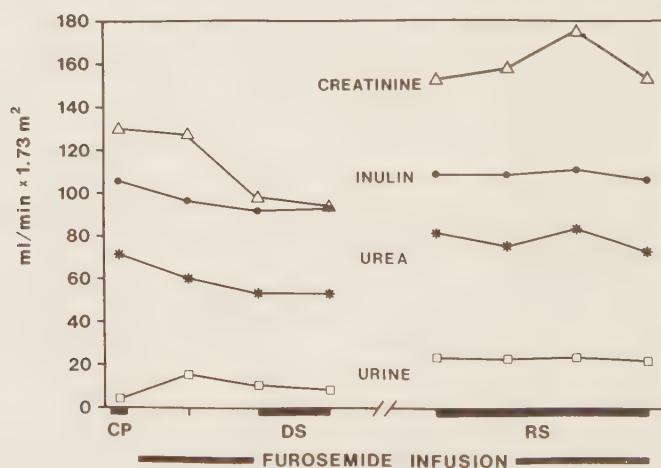


Fig. 18

Mean values of urine flow rate and renal clearance of inulin, creatinine and urea from the control period (CP) and during furosemide infusion through the dehydrated state (DS) to the rehydrated state (RS). (From VI).

GENERAL DISCUSSION

The diuretic response to loop diuretics varied substantially both in the healthy volunteers and in the studied patients with renal disease. This was a result of differences both in the tubular delivery of the drugs to their site of action and in the renal sensitivity to the drugs.

Two types of tolerance could be identified, acute tolerance, developing within hours and related to dehydration (ECV contraction), and delayed tolerance, which appeared after one week's treatment without any signs of dehydration. At least two types of poor response could also be identified, one resulting from decreased delivery and the other from decreased sensitivity (resistance). Decreased renal sensitivity to loop diuretics can be due either to intrarenal mechanisms or to the action of hormonal factors on the kidneys, or both.

Delivery of a loop diuretic to its site of action

Loop diuretics, e.g. furosemide and piretanide, compete with chloride for one of the anion binding sites of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter at the luminal membrane in the thick ascending limb of Henle's loop (TALH) (77, 110). Thus, it is expected that the urinary concentration of (unbound) drug at the receptor sites will be the primary determinant of the diuretic effect. This was shown by Burg et al. (25) in 1973 with use of the isolated perfused tubuli technique. As the tubular concentration of a drug cannot be measured in vivo, the delivery rate of the diuretic to its site of action, which is equivalent to the urinary excretion rate, is used as the "dose" in the dose response curve (28, 90). Furosemide and piretanide are mainly excreted into the tubules by the organic acid transport pathway in the proximal tubule (35, 90, 91). Significant tubular reabsorption of the drugs is unlikely, as pKa of furosemide is 3.8 and that of piretanide 4.1 (91). Thus, the rate of urinary excretion of the drug is a useful approximation of the delivery rate to TALH. The actual concentration in TALH can be estimated from knowledge of the delivery rate, GFR, and the tubular fluid reabsorption in segments proximal to TALH. If

furosemide decreases proximal reabsorption (105), this will decrease the furosemide concentration at the site of action. We found that GFR decreased and proximal tubular fluid reabsorption increased during dehydration in the acute tolerance study (I, V). As the urinary excretion rate of furosemide changed very little in that study (Fig. 20), the urinary concentration of this drug was probably higher at its site of action during dehydration than after rehydration, indicating that mechanisms other than pharmacokinetic ones underlay the poor diuretic response in the dehydrated state.

In nephrotic patients up to 75 % of furosemide in the urine can bind to urinary albumin (118). It has been suggested that such binding decreases the effect of furosemide (52). The proteinuria and increased albuminuria in our nephrotic patients with the albumin protocol did not, however, seem to impair the renal sensitivity to total urinary furosemide.

The pharmacokinetic data of furosemide and piretanide in our studies (Table VI) are in accordance with earlier reports (furosemide: Table I; piretanide: 18, 84).

The rate of excretion of a loop diuretic is determined by the product of its renal clearance and the plasma concentration of the drug. In the acute tolerance study (I) the renal clearance of furosemide increased significantly (54 %) after rehydration, while the metabolic clearance of the drug remained unchanged (Table IV). The change in the renal clearance of furosemide followed the same pattern as that of inulin and PAH. The change in GFR cannot explain the increase in the renal clearance of furosemide, since only 1 - 2 % of plasma furosemide is filtered freely. However, the tubular secretion of furosemide is also restricted to some degree by protein binding (15, 57) and we found a renal extraction of furosemide (Cl_F/Cl_{PAH}) of about 20 % irrespective of the state of hydration. Our study gave an opportunity to evaluate the consequences of altered protein binding for the tubular secretion of furosemide in healthy kidneys. The decrease in plasma albumin (from 48.2 to 40.4 g/l) after rehydration resulted in an increased free fraction of furosemide (f_u). Furthermore, the filtration fraction decreased from 0.26 to 0.20, which meant that plasma albumin changed from 65.1 $[48.2/(1-0.26)]$ to 50.5 $[40.4/(1-0.20)]$ g/l at the site of secretion. As the molar concen-

tration of furosemide in our study was low (2.5 μ M) compared with that of albumin (700 μ M), only the strongest binding site should have been of importance. It was assumed that the binding of furosemide to albumin obeyed the law of mass action. It could therefore be estimated a) that the change in f_u was inversely proportional to that of albumin*, b) that rehydration increased f_u by 19 % and 29 % in pre- and postglomerular blood, respectively, and c) that the renal clearance would have increased correspondingly. However, there was a 54 % increase in renal clearance. Thus, some of this increase must have been due to increased clearance of unbound furosemide, partly caused by a change in the renal blood flow distribution and by increased renal blood flow. There may be differences in furosemide extraction among different nephron populations, and the secretory surface may increase with the blood flow.

The increased renal clearance of furosemide after rehydration resulted in a small but significant increase in the rate of delivery of furosemide to its site of action in the tubule in the rehydrated state as compared with that during dehydration (Table IV). In steady state with continuous infusion of furosemide, the dose fraction excreted unchanged in the urine (calculated as renal/total clearance fraction) would have increased from 53 to 63 % after rehydration. This is contrary to the reports by Branch et al. (17) and Wilcox et al. (131), who found no change in furosemide clearance and delivery after sodium depletion. These groups, however, used single-dose techniques, as opposed to our design with continuous infusion, which gives stable plasma levels of furosemide and conditions for measuring clearance with better precision. Thus, isotonic dehydration reduces the renal clearance of furosemide substantially, but reduces its renal excretion to a lesser degree, and the concentration of the drug at the site of action may even increase (see above).

In our study of nephrotic patients (IV) we managed to change the plasma albumin concentration without altering GFR. As in the acute tolerance study (I), we found an inverse relation between the plasma albumin level and the renal clearance of furosemide (Fig 16A). All

 *) $F + A \leq FA$; $[F] \cdot [A] / [FA] = K$; $r = [F] / [FA]$; $r = K / [A]$;
 $r_1 / r_2 = [A_2] / [A_1]$. r approximates f_u , when $f_u < 0.05$.
 F =furosemide, A =albumin, f_u =free fraction of furosemide.

patients showed higher renal clearance (mean +46 %) with the dextran protocol (P-albumin 23 g/l, giving an estimated 43 % higher free fraction of furosemide) than with the albumin protocol (P-albumin 32 g/l). Likewise, the non-renal clearance of furosemide also changed inversely to the change in plasma albumin (Fig. 16A). Since the extraction of furosemide in the liver and the kidney is low (less than 30 %), owing to extensive protein binding, both clearances are expected to be related to the unbound fraction (109). The low plasma albumin with the dextran protocol, however, also led to an increased volume of distribution of furosemide (Fig. 16B). This meant a shift of furosemide from intra- to extravascular compartments when the plasma albumin concentration fell (Fig. 19). The net result of these opposing effects was an almost unchanged urinary excretion of furosemide. Actually, less furosemide was excreted in the urine during the control day than after albumin infusion. Similar results have been reported previously (73, 118) and in a recent study in albuminaemic rats (68), in which the plasma clearance and distribution volume of furosemide were very high, but the fraction of administered furosemide excreted in the urine was very low.

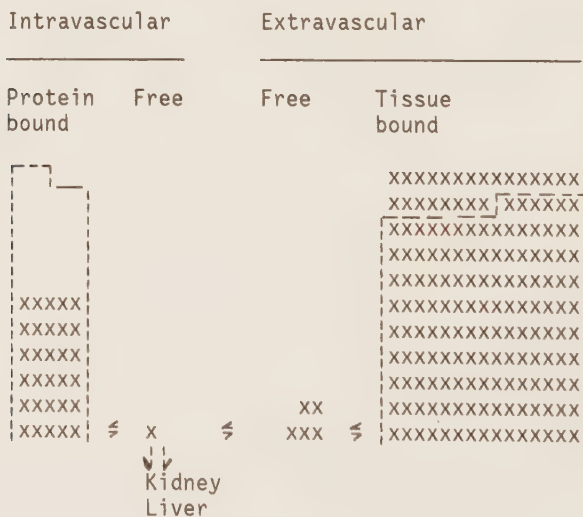


Fig. 19

Distribution of furosemide in the body, calculated from values in Table VI, with protocol D+F with the changes with protocol A+F indicated (||). Note that the concentration of free furosemide does not change with increased plasma albumin, but part of the tissue-bound drug is transferred to plasma albumin.

Thus, in our study of nephrotic patients, as in the acute tolerance study (I), the altered plasma albumin concentration changed the renal clearance of furosemide substantially without affecting the urinary delivery of the drug.

In previous studies in nephrotic patients no significant change in the renal clearance of total furosemide (compared with healthy volunteers) has been found in spite of an increased unbound fraction (73, 106, 118). The effects of low plasma albumin might have been masked by interindividual differences in the renal clearance of furosemide and by differences in renal function, compared with the control group, that were not detected by creatinine clearance. High non-renal clearance was found in two studies (73, 118) and the apparent volume of distribution was raised in two (106, 118). The dose fraction excreted unchanged in the urine was equal to (104, 106) or lower than that in healthy subjects (73, 118).

In the delayed tolerance study (II) there were significant changes in inulin and PAH clearances between the protocols on day 7, but no changes in plasma albumin or in the renal clearance of furosemide. The urinary excretion rate and the urinary recovery of furosemide were also the same in all protocols, on day 1 and day 7 (Fig. 20). Thus, the delayed tolerance to furosemide was not the result of a decrease in the delivery of the drug to its site of action.

In the piretanide study (III) the bioavailability of the drug was found to be very high in the predialysis patients. This is the primary prerequisite for a high urinary delivery from an oral dose and an attractive feature of a loop diuretic. The non-renal clearance was unchanged compared with that in healthy volunteers, but the renal clearance was reduced more than was expected from the residual function (about 50 % of GFR). This is in concordance with reports on reduced renal clearance of furosemide in patients with renal insufficiency, which was explained by competitive inhibition of the tubular secretion by accumulated uraemic metabolites (106, 107). An alternative explanation, however, could be a reduced extraction of piretanide due to fibrosis and architectural alterations of the kidneys (cf. data on PAH extraction in chronic renal failure; 12). Regardless of the true mechanism, this resulted in a low fraction of the dose being excreted unchanged in the urine (healthy volunteers: 49 % of i.v. dose; predialysis patients: 7 % of i.v. dose; dialysis patients 0.8 % of oral

dose). To excrete a comparable amount of piretanide, the plasma concentration of the drug in the predialysis patients was about ten times that in the healthy volunteers, since the renal clearance was one-tenth of theirs. Therefore, in absolute terms ten times more of the drug was "wasted" by non-renal clearance. According to the nephron loss hypothesis, the excretion of piretanide per functioning nephron would be ten times higher than in a normal nephron at the same urinary excretion rate of the drug.

Renal sensitivity

Besides the advantage in obtaining more accurate clearance measurements, when studying tolerance to diuretics it is a further advantage if the excretion rate of the drug can be kept relatively constant, since the efficiency changes with the delivery rate of the drug (58, 71). In single-dose studies there can be substantial changes in the delivery rate within one urinary collection period (Fig. 20). In the acute tolerance study (I), the changes in the furosemide delivery rate were very small (Fig. 20). The renal sensitivity to furosemide, defined as the relation between the urinary sodium and urinary furosemide excretion rates, decreased during dehydration and was restored

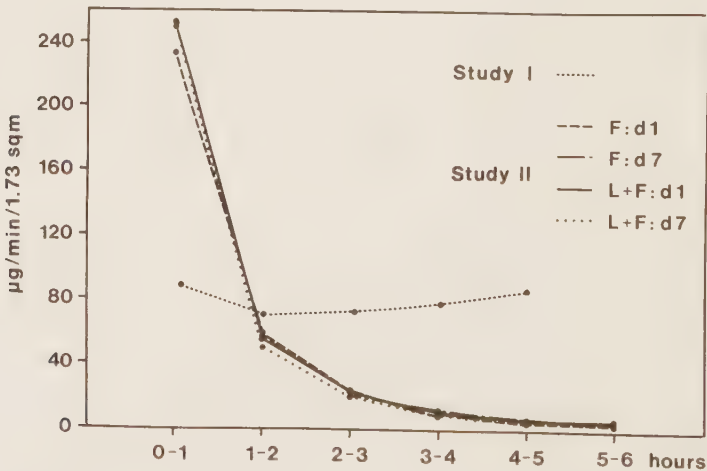


Fig. 20

Urinary furosemide excretion rate up to 6 h after i.v. injection of 40 mg furosemide with the different protocols of study II, compared with that during continuous infusion of the drug at 8 mg/h in study I.

by rehydration (Fig. 21). The changes in sensitivity during dehydration were qualitatively similar to those described by Hammarlund et al. (58) in healthy volunteers after single oral doses of furosemide. As seen in Figure 21, the renal sensitivity was higher with continuous infusion than after a bolus injection of furosemide in studies II and IV.

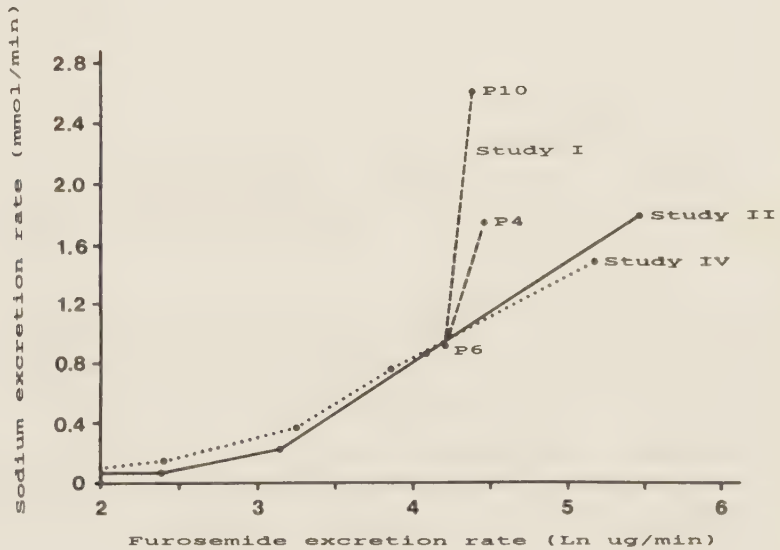


Fig. 21

Comparison of the renal sensitivity to furosemide between studies I, II (F:day 1) and IV (control day).

In the delayed tolerance study (II), single doses were given and the response is illustrated in three ways, 1) by a sensitivity curve, i.e. the chloride excretion rate in relation to the urinary drug excretion rate (Fig. 23), 2) by an efficiency curve in which chloride excretion per amount of furosemide excreted is plotted against different furosemide excretion rates (Fig. 22), and 3) by an efficiency curve in which chloride excretion per amount of furosemide excreted is plotted against time (Fig. 9). Maximum efficiency was noted when the furosemide excretion rate was about 60 ug/min 1-2 h after a dose (cf. Hammarlund -85, 50-100 ug/min; Kaojarern -82, 22 ug/min; refs 58, 71). There was no difference in the furosemide excretion rate between the different protocols (Fig. 20). The efficiency curves plotted against time are therefore comparable and show significant

changes from day 1 to day 7. Thus, the delayed tolerance is altogether an expression of a decrease in renal sensitivity to the diuretic.

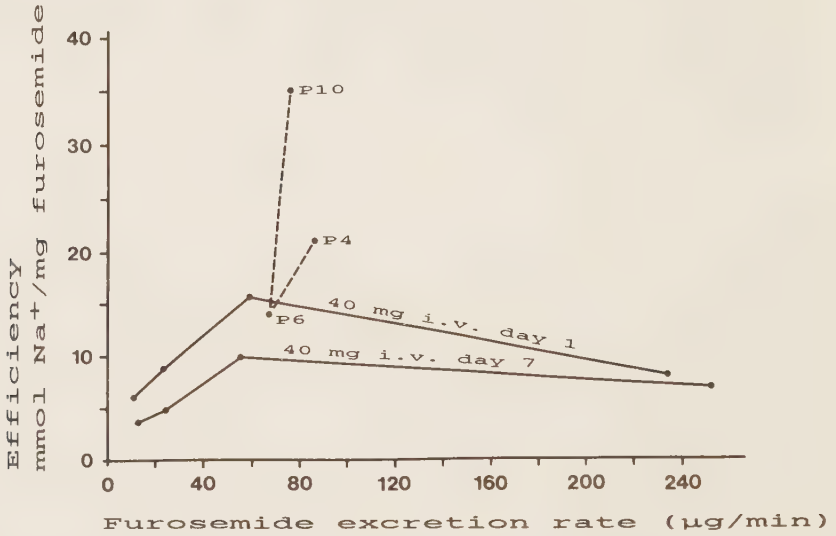


Fig. 22

Efficiency of furosemide plotted against the furosemide excretion rate. Comparison between the acute tolerance study (I) during the start of dehydration (P4), after dehydration (P6) and the delayed tolerance study (II:F protocol), on day 1 and day 7.

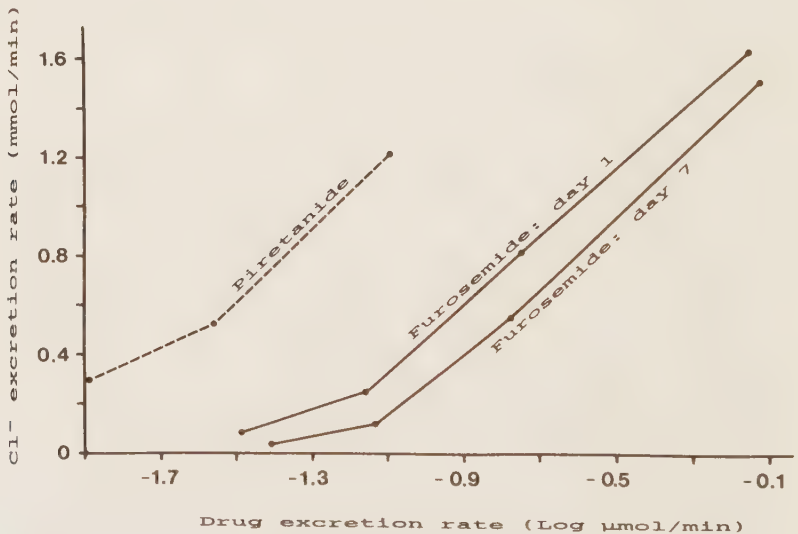


Fig. 23

Comparison of the renal sensitivity of piretanide (study III; healthy volunteers) with that of furosemide in the delayed tolerance study (II; F protocol) on day 1 and day 7. It is seen that an approximately four times higher molar concentration of furosemide is needed to get the same effect.

In the piretanide study there was no strong interindividual correlation between the plasma piretanide level and the chloruretic effect (Fig. 14). The urinary excretion rate vs effect plot (Fig. 15) shows a much closer relationship, even when the dialysis patients are included. This is in accordance with the established knowledge that loop diuretics act from the urinary side of the nephron and that they have little effect from the peritubular side (25, 94). Further, this implies that in spite of severely damaged kidneys and a uraemic condition, the overall renal sensitivity to loop diuretics such as piretanide is normal. In the renal patients the decreased GFR will have meant that they had to increase the excretion fractions of sodium and chloride correspondingly to get a certain saluretic effect. They also showed correspondingly higher piretanide excretion per functioning nephrons (see above).

Fig. 23 shows the difference in the sensitivity of healthy volunteers to piretanide (III) and furosemide (protocol F:d1 and F:d7 in II). An approximately four times higher molar excretion rate of furosemide than of piretanide is needed to get the same effect. This relation is similar to the findings of Schlatter et al. (110) when they compared IC_{50} of piretanide and furosemide in a study of the $Na^+/K^+/2Cl^-$ cotransporter in the rabbit cortical TALH (p. 11), i.e. on a receptor level.

Dose-effect curves are normally S-shaped, with an upper plateau at which a further increase in dose (excretion rate of a diuretic) does not enhance the effect. The highest doses of piretanide in our uraemic patients were above the plateau level (Fig. 13) and a substantial part of the dose was consequently "wasted". Thus, dividing a high daily dose of piretanide into repeated doses of 24 mg (near maximal response) would increase the efficiency in the uraemic patients. In patients with creatinine clearance of less than 20 ml/min, Brater et al. (20) found that 160 mg of furosemide or 4 mg of bumetanide is enough to reach the upper plateau of the dose response curve, and concluded that there is no justification for administering larger single doses to such patients.

Only one out of five of the nephrotic patients in our study (IV) showed a markedly decreased sensitivity to furosemide. This was also the only patient with hypovolaemia and greatly increased plasma renin

activity and plasma aldosterone. As a group, however, the sensitivity to furosemide in the nephrotic patients was not lower than that in healthy volunteers (Fig. 21). This means that when treating oedema with a loop diuretic in nephrotic patients, it is wise to start with a normal dose and increase it if necessary. Volume expansion with dextran/albumin may have caused a small increase in the renal sensitivity to furosemide in our nephrotic patients (Fig. 17).

Mechanisms underlying tolerance:

The decreased response to furosemide during dehydration (I) and after one week's treatment (II) indicated that tolerance was induced. The changes in furosemide delivery induced by dehydration were small compared with the change in the diuretic response. From the dehydrated to rehydrated state the furosemide excretion rate increased by 20 %, at the same time as the urine flow and the natriuresis increased by about 150 %. One week's treatment induced no dehydration or plasma albumin changes and no change in furosemide clearance or urinary delivery.

ECV contraction is a strong stimulus to RAAS and the sympathetic nervous system (p. 10). Accordingly, we found signs of activation of both systems after dehydration in the acute study (I), such as elevated plasma levels of PRA, aldosterone and norepinephrine (but not of epinephrine), a decrease in conjunctival oxygen tension, reflecting a transient decrease in capillary blood flow (2), and increased renal vascular resistance. The main cause of the acute tolerance, increased proximal and distal tubular reabsorption (V), could have been a consequence of renal sympathetic nerve and RAAS stimulation (9, 69, 136). The acute tolerance could be rapidly reversed by rehydration, a finding that does not contradict a neuro-hormonal mechanism. In our experimental model, inhibition of the sympathetic nervous system and of RAAS would reveal the relative importance of each to the development of acute tolerance. In previous studies, with fully hydrated subjects, neither angiotensin converting enzyme inhibition nor alpha-adrenoceptor blockade altered the response to a single intravenous dose of furosemide (74, 132). Of importance to the present discussion is the fact that in an earlier study using the same design, only the rebound phenomenon (see below) was observed, but no tolerance to furosemide (131).

AVP and plasma osmolality were unchanged during dehydration/rehydration and therefore do not seem to be involved in the development of acute tolerance to furosemide diuresis.

In the delayed tolerance study (II) no stimulus was provided by ECV contraction. If there was slight RAAS activation before the furosemide dose on day 7, which could neither be verified nor ruled out, the effects should have been adequately inhibited by lisinopril, but the same tolerance development was found when furosemide was administered with as without lisinopril.

Thus, acute tolerance seems to be related to dehydration, and possibly mediated by RAAS and/or the sympathetic nervous system. Delayed tolerance, on the other hand, does not seem to be associated with dehydration, indicating involvement of other factors.

Some intrarenal mechanism for ECV preservation, e.g. resetting of the tubuloglomerular feedback mechanism (13) or changes in the colloid osmotic (21) and hydrostatic pressure gradients over the tubule (112), could have contributed to both types of tolerance phenomenon. Such mechanisms cannot, however, be studied directly in humans. For example, during dehydration in the acute tolerance study (I) the plasma albumin concentration and filtration fraction increased, resulting in an increase in the postglomerular albumin concentration to up to 30 % above the concentration in the rehydrated state (see above). Elevated peritubular plasma oncotic pressure would have increased the proximal tubular reabsorption (21) and could have contributed to the acute tolerance phenomenon.

Concerning delayed tolerance, structural adaptation may develop in the kidney after one week of treatment. Furosemide has been shown to increase Na^+/K^+ -ATPase activity in the cortical and medullary collecting duct in rats, indicating an increased capacity for sodium transport (42, 45). Structural adaptation of the distal convoluted tubule, such as tubular cell proliferation and differentiation and increased basolateral and luminal membrane areas has been observed in rats after six days of furosemide treatment (70). It has recently been demonstrated in rats that this structural adaptation is associated with an increase in the transport capacity of the distal convoluted tubule after six days of furosemide infusion and a high-salt diet (41). Thus, after one week of furosemide treatment an intrarenal adaptation could have occurred so that nephron segments distal

to the site of action of the drug could have increased their capacity to reabsorb salt and water. A decreased urinary Na^+/K^+ ratio from day 1 to day 7 (data not shown) is an indication of increased distal sodium reabsorption, whether the cause is structural adaptation or secondary hyperaldosteronism.

Another possibility is that the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter, the "furosemide receptor" (54), could have increased in number or effectiveness or that its sensitivity to furosemide could have been decreased after one week (or more rapidly as in the acute tolerance study) of furosemide treatment. In Ehrlich ascites tumour cells the transporter is activated after shrinkage of the cells and almost completely inactivated at a certain cell volume (64). A similar finding has been reported recently for the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter in isolated rabbit kidney cells from the thick ascending limb of Henle's loop (44).

Results of the present studies demonstrate that acute tolerance develops in direct relation to dehydration. Activation of RAAS and the sympathetic nervous system may contribute to the acute tolerance. By contrast, the delayed type of tolerance to furosemide diuresis is unrelated to dehydration and most likely is not due to RAAS activation. Other mechanisms, probably intrarenal, will have to be looked for in both types of tolerance; in the delayed tolerance even structural adaptation.

Mechanisms underlying resistance

The pathophysiological mechanisms of salt retention in oedematous disorders are probably the main factors responsible for diuretic resistance to treatment with diuretics.

The exact nature of such mechanisms in nephrotic patients has been much debated in recent years. Proteinuria and hypoalbuminaemia are central features, but hypovolaemia is not a constant finding (36). Some other mechanisms underlying salt retention than RAAS activation, probably intrarenal, are indicated (23, 29, 67, 81). In our nephrotic patients plasma volume expansion with albumin or dextran suppressed the plasma aldosterone concentration but had only minimal influence on ANP.

Initially the diuresis increased but not the saluresis, in accordance with previous findings in nephrotic patients (22, 80) and in healthy

volunteers (5).

Decreased renal sensitivity was found only in the one patient with hypovolaemia and activated RAAS. The plasma volume expansion increased the renal sensitivity to furosemide only slightly both in the normovolaemic and the hypovolaemic patients, despite suppression of RAAS activity. This indicates that other mechanisms than hypovolaemia and RAAS activation are responsible for salt retention and diuretic resistance in nephrotic patients.

The presence of albumin within the tubular lumen may in itself in some way increase tubular sodium reabsorption (67). Furthermore, albuminuria may decrease the sensitivity to furosemide by binding the drug to protein (52, 118). We could not exclude the possibility that the increased albuminuria after albumin infusion may have had slight influence (Fig. 17). A comparison with healthy volunteers (Fig. 21) indicates, however, that urinary protein binding has very little impact on the renal sensitivity to furosemide.

In the nephrotic patients the delivery was almost normal in spite of substantial changes in many pharmacokinetic parameters (see above). The reduced response to piretanide in the patients with chronic renal failure was entirely due to decreased urinary delivery of the drug. The overall renal sensitivity was normal. A low GFR, however, limits the maximal response.

The rebound phenomenon

The compensatory mechanism underlying tolerance is still operative after the acute diuresis caused by the diuretic has subsided, resulting in relative retention of salt and water in the subsequent period. This is commonly referred to as a "rebound effect". Such an effect was evident in the delayed tolerance study both on day 1 and day 7 (Fig. 8) and in the piretanide study (Fig. 12). When salt intake was unrestricted during treatment with furosemide, we found that the overall salt balance was maintained by the delayed tolerance and rebound phenomenon. The natriuretic response to furosemide after 2 days of treatment is, however, unaltered (131). Using treatment with captopril and prazosin, Wilcox et al (132) found that RAAS and the sympathetic nervous system are not essential for the rebound effects.

Methodological aspects

In diuretic studies the urinary flow rate changes, and so does the tubular flow rate and proximal and distal tubular reabsorption. Therefore, a filtration marker which is secreted and/or reabsorbed in the tubule will mislead the investigator more in diuretic studies than otherwise. Creatinine is an unreliable marker of GFR (VI), as evident by the fact that the creatinine to inulin clearance ratio changed from 1.05 to 1.47 between the states of dehydration and rehydration, indicating that there is varying tubular secretion and reabsorption of creatinine according to the degree of hydration (Table VII). In the calculations of tubular reabsorption ($1 - EF$) in the acute tolerance study (I), depicted in Figure 24 A and B, inulin and creatinine, respectively, were used as filtration markers. Creatinine gives higher values of tubular reabsorption and less change between dehydration and rehydration, since the tubular secretion and reabsorption of creatinine change in the same directions as the reabsorption of sodium and lithium. In study IV the use of creatinine as a filtration marker (Fig. 25) would have falsely indicated an increase in GFR after volume expansion with albumin (A) and after administration of furosemide (C+F and A+F). Thus, if creatinine were to be relied on as a marker of GFR, altered tubular reabsorption underlying tolerance effects of diuretics and as a cause of effects of plasma volume expansion would be difficult to detect and the effects would wrongly be attributed to a change in GFR.

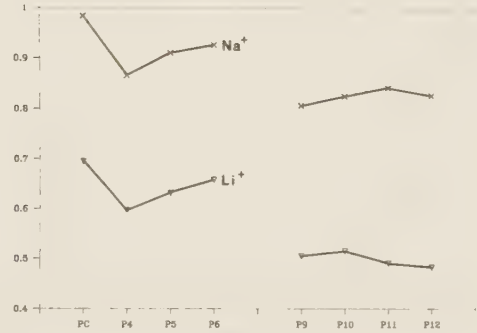
Segmental sodium reabsorption by the renal tubule is of great interest in diuretic studies. There are, however, no ideal markers that can readily be used to quantify the reabsorption in different tubular segments. Urea clearance has been shown to decrease with decreased urine flow and increased proximal reabsorption and in that respect urea is an indicator of proximal reabsorption (50).

Lithium (Li^+) may be the best marker of proximal tubular sodium (chloride and water) reabsorption (122, 123) and has been used frequently in the last few years, even in diuretic studies (30, 108). The prerequisite for the use of Li^+ as such a marker is that it is reabsorbed exclusively in the proximal tubule and in the same proportion as sodium. The excretion fraction of Li^+ (EF_{Li}) would in that case be an estimate of the fraction of filtered Na^+ delivered to the loop of Henle (called "proximal EF"). $1 - EF_{Li}$ would indicate

proximal tubular Na reabsorption and EF_{Na}/EF_{Li} the Na excretion fraction of the nephron distal to the "Li reabsorption area", called "distal EF".

Fig. 24

A. Tubular reabsorption of Na^+ and Li^+ ($1-Cl_{Na}$ or Li/Cl_{inulin}) in the acute tolerance study (I), with inulin used as a filtration marker.



B. The same as A, but with creatinine instead of inulin as a filtration marker.

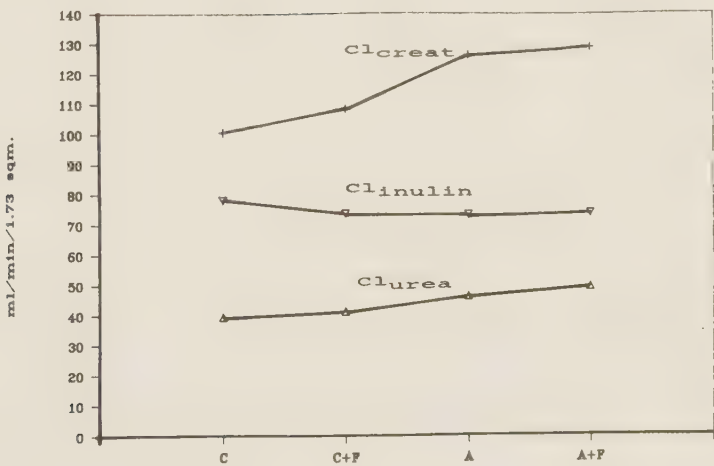
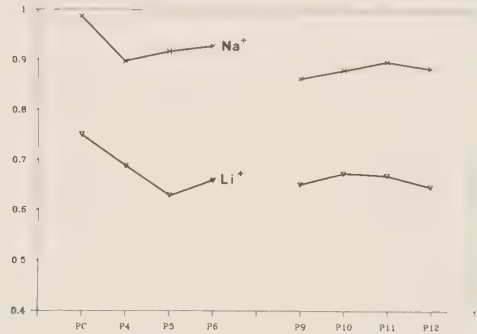


Fig. 25

Mean clearance values of inulin, creatinine and urea in study IV during the control period (C), with furoseamide (C+F) and after plasma volume expansion with albumin (A) and with furoseamide (A+F).

It should be noted that "distal EF" is the excretion fraction of a nephron segment, a segmental EF (SEF; cf. Table I), i.e. a fraction of the amount delivered to the segment. The customary way of describing transport in a nephron segment is as fractional reabsorption (SFR) related to filtered amount (49, 75). The sum of SFR for all nephron segments gives the fractional reabsorption of the nephron (Table I). SEF is a more physiological and functional parameter for describing transport in a segment, as here the transport is related to the amount delivered to the segment and SEF is an exponential function of the segmental passage time (related to tubular flow rate). Moreover, the product of SEF for all nephron segments gives the nephron EF (Table I). The urinary sodium excretion rate ($U_{Na} \cdot V$) is determined by the product of GFR, EF_{Na} and P_{Na} . EF_{Na} can be divided into an arbitrary number of SEF. This makes it possible to describe the significance of the effect of a diuretic on each of the above factors, since the proportional change compared with the control value of each factor indicates its relative importance for the natriuretic effect, given that the other variables are unchanged. For example in the acute tolerance study (I and V) the following results were obtained (Table VII):

$U_{Na} \cdot V$	=	GFR	*	EF_{Li} Prox.	*	$[EF_{Na}/EF_{Li}]$ Dist. EF	*	P_{Na}	Total
CP		106		0.30		0.05		137	218
P4		96		0.40		0.34		137	1789
P4/CP		0.91		1.33		6.80		1	8.20
Change %		-9		+33		+580		0	+720

The initial effect of furosemide on GFR would have reduced natriuresis by 9 %. According to Thomsen's hypothesis (123), the effect of furosemide on the proximal tubule would have increased natriuresis by 33 %, and the corresponding increase due to the effect of furosemide on the distal tubule would be an increase by 580 %, altogether an increase of 720 %. As the different factors interact, the total effect is greater than the sum of the parts. These figures are reasonable; the "proximal" effect is moderate compared with the "distal".

Tubular reabsorption of Li^+ (Fig. 24 A) would refer to the proximal tubular reabsorption. There are two possible interpretations of the initial effect: 1) that furosemide has a proximal effect, which is controversial (105, 126) and 2) that Li^+ is reabsorbed distally by a transport mechanism inhibited by furosemide, probably in TALH (by the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter or by the electrochemical gradient). In accordance with our findings, a substantial increase in EF_{Li} has been found after administration of furosemide (30, 108, 120). The decrease in EF_{urea} (Table VII) in spite of an increased urine flow indicates that the proximal reabsorption may even be increased in the initial period (50). This may be true, as the plasma volume had already decreased by 11.5 % in P4, but in that case Li^+ failed as a marker of proximal reabsorption. Thus, the most likely interpretation of our results is that Li^+ also has a more distal site of reabsorption, which could be inhibited by furosemide. EF_{Li} would then have underestimated "proximal EF" during the control period. Thomsen et al (124), observed in fact, that a low sodium diet in rats induced distal Li^+ reabsorption, which could be abolished by amiloride and furosemide.

The changes in EF_{Li} during continuous furosemide infusion (P4-P12) are more reliable and should be so if distal reabsorption of Li^+ is blocked by furosemide. The increase in tubular reabsorption of Li^+ during dehydration and the decrease after rehydration (Fig. 24 A) are changes that are expected for proximal tubular reabsorption. Neither of these changes in proximal tubular reabsorption can be revealed with creatinine as a marker of GFR and Li^+ as a marker of proximal tubular reabsorption.

Thus, we found that Li^+ is an indicator but not an ideal marker of proximal tubular sodium reabsorption. Our results suggest that there is furosemide sensitive distal Li^+ reabsorption. Li^+ should therefore not be used to determine the site of action of furosemide or to follow changes in proximal reabsorption during furosemide diuresis. It is proposed that the lithiuretic effect of furosemide may be useful in Li^+ intoxication provided that the patient is not dehydrated or hyponatraemic.

SUMMARY AND CONCLUSIONS

From the results of this investigation the following conclusions regarding reduced effects of loop diuretics can be drawn:

- I:
 - a. Isotonic dehydration induced acute tolerance to furosemide, which was rapidly reversed by rehydration.
 - b. Dehydration decreased the renal clearance of furosemide substantially, mainly through an increase in the plasma albumin concentration, which decreased the free fraction of furosemide.
 - c. Nevertheless, only minimal changes in the urinary furosemide delivery rate were found. Thus the acute tolerance is not due to pharmacokinetic changes.
 - d. In association with dehydration, the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) were activated, which may have contributed to the markedly reduced renal sensitivity to furosemide under this condition. Intrarenal mechanisms for preservation of the extracellular fluid volume could also have contributed.
- II:
 - a. A delayed type of tolerance to furosemide was evident after one week of treatment.
 - b. The urinary delivery of furosemide was unchanged.
 - c. Angiotensin converting enzyme inhibition did not affect this decrease in renal sensitivity to furosemide, which was unrelated to dehydration and most likely not due to RAAS activation. Other mechanisms, probably intrarenal (structural adaptation?) therefore, have to be looked for.
- III:
 - a. The renal sensitivity to piretanide in healthy volunteers was found to be four times higher than that to furosemide.
 - b. The non-renal clearance of piretanide was unchanged in patients with renal disease, but the renal clearance was reduced more than could be expected from GFR, resulting in a low fraction of the dose excreted unchanged in the urine.

- c. Submaximal doses of 24 mg of piretanide twice or three times daily are recommended for uraemic patients, since higher doses lead to "waste" of drug with no added effect.
- d. The renal sensitivity to piretanide (effect in relation to urinary excretion of drug) was unchanged in patients with chronic renal failure. Thus, there is a purely pharmacokinetic mechanism underlying the resistance in patients with chronic renal disease.

IV: a. A decrease in the plasma albumin concentration increased the renal clearance of furosemide significantly but did not increase the urinary delivery of the drug.

- b. Plasma volume expansion caused no increase in the urinary sodium excretion and only a minor increase in the renal sensitivity to furosemide.
- c. Only one of five nephrotic patients was resistant to furosemide. This patient was hypovolaemic and showed signs of activated RAAS. Correction of the hypovolaemia blocked the RAAS activation, but the resistance to furosemide persisted. This resistance was therefore probably intrarenal.
- d. The sensitivity to furosemide was not decreased in the other four patients.

V: a. Creatinine is an unreliable marker of GFR because of its substantial tubular secretion and reabsorption, which are related to the degree of hydration.

- b. Li^+ is an indicator of proximal tubular sodium reabsorption but not an ideal marker of reabsorption at this site, as furosemide-sensitive distal tubular reabsorption of Li^+ has been found to take place.

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